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Texture analysis of pharynx in chronic phase Epstein-Barr virus infection

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Abstract

Aim: Epstein-Barr virus (EBV) is a human herpes virus that can be found worldwide and causes latent infections. EBV is responsible for the etiology of infectious mononucleosis, Burkitt's lymphoma, and nasopharyngeal carcinoma (NPC) through the transformation of infected cells. In this study, it was aimed to investigate nasopharyngeal soft tissue differentiation by texture analysis in neck computed tomography (CT) images of EBV-positive individuals.

Material and Methods: EBV-positive and negative healthy individuals of the same age and gender were selected consecutively from HIS and their neck CT images were evaluated with texture analysis.

Results: While there was no significant difference between the groups in any of the same tissue parameters investigated for the largest lymph node in the neck, it was observed that only a small fraction of the 224 tissue parameters analyzed for each of the largest lymph node at the right neck IIA level, pharynx, and nasopharynx were statistically significant.

Discussion: Despite some limitations, texture changes on CT in chronic EBV infection may lead to the identification of imaging markers that predict individuals at high risk of NPC.

Keywords

Human Herpesvirus 4, Epstein-Barr Virus Infections, Computerized Tomography, Computer-Assisted Image Processing, Nasopharyngeal Carcinoma

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Introduction

Epstein-Barr virus (EBV) is a human herpes virus that can be found worldwide, causing lytic and latent infections and transformation of infected cells [1]. Serological and nucleic acid hybridization methods have shown that EBV is responsible for the etiology of infectious mononucleosis (IM), Burkitt's lymphoma (BL), and nasopharyngeal carcinoma (NPC). The virus has two target cell types, these are B lymphocytes and epithelial cells. The virus replicates in vivo in the lymphoid and nasopharyngeal tissue epithelium [2-4]. EBV, which can be found in the saliva of 10-20% of normal people, is transmitted by close contact (kissing disease) and enters the body through the oropharyngeal mucosa. EBV first infects the epithelial cells of the oropharynx and salivary glands, and then the lymphoid tissues of the larynx. Heterophile antibodies to EBV infection, usually subclinical, are found in 90% of people over the age of four, regardless of gender. IM is common worldwide and usually occurs in the 15-20 age group, while BL is more common in children from Central Africa and New Guinea, and NPC is more common in males living in South East Asia. Serology and virus isolation can be used for diagnosis. There is no specific treatment for EBV infection [5-7].

The contents of the pharyngeal soft tissue include the mucosa of the nasopharynx and oropharynx, as well as the submucosal structures surrounding Waldeyer's ring, minor salivary glands (MSG), pharyngeal constrictor and levator veli palatini muscles, and the torus tubarius. The Waldeyer ring includes the adenoids, palatine, and lingual tonsils, and the ring-like configuration of lymphoid tissue can be considered as a mechanism that protects the body from inhaled and ingested antigens. Tonsil crypts lined with deep mucosa cause a characteristic striated appearance on contrast imaging and may obscure small primary tumors [8-10]. Squamous cell carcinoma originating from the mucosa represents the most common malignant neoplasm in pharyngeal cavity. Tumors originating from Waldeyer ring lymphoid tissue and MSG are extremely rare. Inflammatory disease of the oropharynx represents a spectrum from non-focal tonsillitis to tonsilar or peritonsillar abscess. Congenital lesions include nasopharyngeal Tornwaldt cysts and those related to the embryological thyroglossal duct, including cysts and lingual thyroid [8, 11-13].

Texture analysis, which is frequently used to identify various biomarkers and characterize tumors and can distinguish between normal and abnormal structures, requires calculating the intensities in the region of interest (ROI) from all pixels using a full statistical analysis [14-17].

In this study, it was aimed to investigate whether the pharyngeal soft tissue differentiates by texture analysis of the neck computerized tomography (CT) images of EBV-positive (case) and negative (control) healthy individuals.

Material and Methods

The entire study was performed in accordance with the 1983 revised version of the 1975 Declaration of Helsinki. Local Ethics Committee approval was obtained (Session Date: 18 November 2021, Issue: 2021/12-03).

Study population

Cases who underwent serological EBV test in the last five years from the University Hospital Information System (HIS) were scanned and 50 cases with neck CT images in the Hospital Picture Archiving and Communication System (PACS) were included in the study. EBV- positive (case group) and negative (control group) healthy individuals of the same age and gender were selected consecutively using HIS and PACS. The cases whose CT image quality was found to be visually low by a 20-year-experienced radiologist (M.B.) were excluded from the study.

Data acquisition and analysis

Real-time polymerase chain reaction (RT-PCR), high sensitivity optimized for diagnostic use, and highly specific QIAGEN artus EBV PCR developed for use with QIAsymphony® SP/AS and Rotor-Gene® Q (QIAGEN GmbH, Hilden, Germany) instruments kits (QS-RGQ Kit) were administered to the subjects using the methods described in the literature [18].

Computerized tomography images were obtained from the 128-slice GE Revolution HD system (General Electric, Milwaukee, WI, USA) installed in the hospital radiology department. Images were transferred from the PACS to a separate storage medium in DICOM (Digital Imaging and Communications in Medicine) image format. Then the images were transferred to a Windows 10 (Microsoft Corporation, Seattle, WA, USA) based computer and processed to obtain the final data. The entire analysis algorithm applied to all selected images was done with an in-house software coded in MATLAB (version R2021b; MathWorks, Natick, MA, USA).

All regions of the pharynx, nasopharynx, and largest in neck and largest in right level IIA lymph nodes were selected individually in the axial images that best represent the anatomy, without exceeding their borders, with an ROI determined by a senior radiologist (M.B.).

Histogram, co-occurrence matrix with all neighbor levels from one to five and run-length matrix values obtained from ROIs have been previously described in the literature [14, 19-21].

Statistical analysis

Data were presented as mean ± standard deviation. The statistical analyses were conducted with IBM SPSS (for Windows version 26, IBM Corporation, Armonk, New York, USA) statistics software. The normality of the distribution of the data was analyzed with the Kolmogorov–Smirnov test. Based on the test findings, Student's t-test and Mann-Whitney U test were used to compare the groups. $p<0.05$ was accepted as statistically significant.

Ethical Approval

Ethics Committee approval for the study was obtained.

Results

There was no statistically significant difference in terms of gender ($p=0.614$) and age ($p=0.878$) between the groups consisting of eight EBV-positive case groups of four people of each gender (mean age, 53.75±16.98 years) and the control group consisting of five females and three males (mean age, 52.88±14.36 years).

It was observed that only a small portion of the 224 texture parameters analyzed for each of the pharynx, nasopharynx, and the largest lymph node at the right neck IIA level were statistically significant ($p<0.05$) (Table 1-3). There was no

Table 1. Statistically significant parameters among those investigated for pharynx.

	Control		EBV		p
	Mean	Standard Deviation	Mean	Standard Deviation	
GLCM - Mean Local Entropy	2.57	0.19	2.85	0.28	0.036*
GLCM - 'Haralick' - '2' - Variance	47.90	3.50	51.55	3.26	0.048*
GLCM - 'Haralick' - '3' - Variance	48.54	3.70	52.37	3.27	0.046*
GLCM - 'Haralick' - '4' - Sum Average	12.36	0.66	13.32	0.91	0.031*
GLCM - 'Haralick' - '4' - Sum Variance	154.59	14.49	175.81	21.71	0.037*
GLCM - 'Haralick' - '4' - Information Measure of Correlation I	-0.1257	0.0751	-0.0587	0.0422	0.045*
GLCM - 'Haralick' - '4' - Maximal Correlation Coefficient	0.3170	0.0952	0.2084	0.1179	0.046†
GLCM - 'Haralick' - '5' - Energy Angular Second Moment	0.3652	0.0581	0.4329	0.0589	0.036*
GLCM - 'Haralick' - '5' - Sum Average	9.24	0.59	10.8	0.69	0.020*
GLCM - 'Haralick' - '5' - Sum Variance	113.74	14.82	133.11	15.77	0.024*
GLCM - 'Haralick' - '5' - Entropy	13.337	0.1274	11.642	0.1752	0.046†
GLCM - 'Haralick' - '5' - Maximal Correlation Coefficient	0.3141	0.0643	0.2157	0.0788	0.016*
GLRLM - '45°' - Short Run Emphasis	0.5893	0.0201	0.6081	0.0226	0.031†
GLRLM - '45°' - Long Run Emphasis	27.28	1.29	26.8	1.44	0.031†

*Student t, †Mann-Whitney U, GLCM = Gray Level Co-Occurrence Matrix, GLRLM = Gray Level Run-Length Matrix

Table 2. Statistically significant parameters among those investigated for nasopharynx.

	Control		EBV		p
	Mean	Standard Deviation	Mean	Standard Deviation	
Histogram - Range	77.13	21.17	182.13	127.10	0.009†
Histogram - Size %L	16.8	2.48	11.46	3.62	0.010*
Histogram - Size %M	68.48	3.82	78.41	9.3	0.012†
Histogram - Size %U	15.44	4.13	9.13	5.80	0.012†
Histogram - Kurtosis	3.4	0.65	11.60	11.46	0.012†
Histogram - Skewness	-0.0659	0.5306	-16.693	17.402	0.027†
Histogram - Root-Sum-of-Squares Level	15157.17	2186.69	21369.41	6010.33	0.046†
GLCM - Contrast	47.24	17.27	185.05	252.64	0.012†
GLCM - Energy	0.0053	0.0013	0.0031	0.0017	0.011*
GLCM - Homogeneity	0.2490	0.0365	0.1902	0.0441	0.011*
GLRLM - '0°' - High Gray-Level Run Emphasis	78.58	19.6	206.79	119.41	0.002†
GLRLM - '0°' - Short Run High Gray-Level Emphasis	10.89	5.64	55.67	89.19	0.009†
GLRLM - '0°' - Long Run High Gray-Level Emphasis	4410.56	1120.96	9877.90	5078.21	0.005†
GLRLM - '45°' - Run Length Nonuniformity	29.27	7.32	54.36	27.14	0.027†
GLRLM - '45°' - Run Percentage	0.4788	0.0928	0.6535	0.2378	0.046†
GLRLM - '45°' - Low Gray-Level Run Emphasis	0.3454	0.0524	0.2702	0.0728	0.033*
GLRLM - '90°' - Run Length Nonuniformity	18.38	3.15	36.97	23.13	0.009†
GLRLM - '135°' - Run Length Nonuniformity	28.17	7.31	47.87	24.73	0.049*

*Student t, †Mann-Whitney U, GLCM = Gray Level Co-Occurrence Matrix, GLRLM = Gray Level Run-Length Matrix

significant difference between groups in any of the same texture parameters investigated for the largest lymph node in the neck ($p>0.05$).

Discussion

Most nasopharyngeal carcinomas (NPC) detected in endemic areas of the world such as southern China, as well as in many non-endemic regions of the world, are undifferentiated carcinomas due to EBV infection. EBV-based markers can be found in blood as well as histological samples of NPC and are used to screen patients. Despite the high EBV-DNA specificity of the population, most patients referred for endoscopic examinations do not have NPC [22, 23].

While plasma EBV-DNA levels remain consistently high in chronic/occult infections, it has been reported that they return to normal after approximately 200 days at the latest in acute EBV-related diseases. The putative potential link between EBV infection and NPC development may provide an imaging marker for the future when CT texture analysis is used [24, 25].

In this study, all eight participants in the case group were still positive for plasma EBV-DNA 2 years after initial infection. It is interesting that EBV-DNA positive participants have different texture characteristics on CT examination than normal ones. Although it is an expected finding to see that EBV infection affects tissue with nasopharyngeal soft tissue involvement, it can be said that the inhomogeneity caused by complex anatomical structures that prevent more tissue parameters from forming differently cannot be excluded with CT. In addition, although the level 2A lymph node shows EBV-related tissue differences, the largest lymph node in the neck does not

Table 3. Statistically significant parameters among those investigated for Lymph node (Level IIA).

	Control		EBV		p
	Mean	Standard Deviation	Mean	Standard Deviation	
Histogram - Root-Sum-of-Squares Level	8923.87	1734.09	11579.39	2792.22	0.038*
GLCM - 'Haralick' - '3' - Difference Entropy	0.5740	0.0502	0.4913	0.0932	0.044*
GLCM - 'Haralick' - '3' - Information Measure of Correlation I	-0.0453	0.0116	-0.0234	0.0216	0.024*
GLCM - 'Haralick' - '3' - Information Measure of Correlation II	0.2281	0.0430	0.1330	0.0745	0.007*
GLCM - 'Haralick' - '3' - Maximal Correlation Coefficient	0.1499	0.0329	0.0946	0.0462	0.015*
GLCM - 'Haralick' - '4' - Energy Angular Second Moment	0.5000	0.0704	0.5944	0.1004	0.047*
GLCM - 'Haralick' - '4' - Contrast	17.63	5.72	12.11	3.67	0.037*
GLCM - 'Haralick' - '4' - Correlation	-0.1644	0.0556	-0.0935	0.0519	0.019*
GLCM - 'Haralick' - '4' - Variance	52.17	2.79	55.48	2.78	0.033*
GLCM - 'Haralick' - '4' - Homogeneity Inverse Difference Moment	0.6473	0.1143	0.7578	0.0735	0.037*
GLCM - 'Haralick' - '4' - Difference Variance	17.2	5.51	11.69	3.54	0.037*
GLCM - 'Haralick' - '4' - Information Measure of Correlation I	-0.1069	0.1088	-0.0272	0.0171	0.005†
GLCM - 'Haralick' - '4' - Information Measure of Correlation II	0.3227	0.1246	0.1459	0.0668	0.003*
GLCM - 'Haralick' - '4' - Maximal Correlation Coefficient	0.2116	0.0830	0.0973	0.0434	0.004*
GLCM - 'Haralick' - '5' - Sum Variance	114.10	28.23	139.23	16.19	0.046*
GLRLM - '0°' - Gray-Level Nonuniformity	3.77	0.62	4.53	0.76	0.045*
GLRLM - '0°' - Long Run High Gray-Level Emphasis	1757.60	623.58	2995.08	1338.72	0.033*
GLRLM - '45° - High Gray-Level Run Emphasis	15.21	4.90	24.27	10.44	0.043*
GLRLM - '45°' - Long Run High Gray-Level Emphasis	915.32	301.82	1479.22	640.03	0.041*
GLRLM - '90°' - Gray-Level Nonuniformity	3.63	0.71	4.51	0.77	0.032*
GLRLM - '90°' - High Gray-Level Run Emphasis	29.73	11.2	49.26	21.50	0.038*
GLRLM - '90°' - Long Run High Gray-Level Emphasis	1781.32	674.67	2990.98	1323.31	0.037*
GLRLM - '135°' - High Gray-Level Run Emphasis of	14.66	5.32	24.22	10.45	0.037*
GLRLM - '135°' - Long Run High Gray-Level Emphasis	884.09	325.25	1476.37	640.59	0.035*

*Student t, †Mann-Whitney U, GLCM = Gray Level Co-Occurrence Matrix, GLRLM = Gray Level Run-Length Matrix

show this relationship. It can be said that EBV infection takes place in the adjacent lymphatic system in addition to nasopharyngeal soft tissue involvement and is limited. In this context, since latent EBV infection plays a role in NPC oncogenesis, texture analysis findings may be a biomarker to pre-identify individuals at risk of developing NPC.

Limitations

The present study had a few limitations. First, the number of cases is very small in order to provide sufficient evidence. Then, other potential factors such as air pollutants and other irritant infections that may be important in the heterogeneity of soft tissues were not analyzed. Third, the pharynx and nasopharynx consist of a large number of tissues that are indistinguishable on CT, making it difficult to obtain a homogeneous texture. Finally, the absence of similar previous studies eliminated the possibility of any comparison.

Conclusion

In the presence of positive plasma EBV-DNA, the presence of texture difference on CT may be an independent predictive factor for NPC. However, these are only preliminary results, this potential link between EBV infection and texture on CT deserves further investigation as it may lead to the identification of imaging markers that predict individuals at high risk of NPC.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

References

1. Epstein MA, Achong BG, Barr YM. Virus Particles in Cultured Lymphoblasts from Burkitt's Lymphoma. Lancet. 1964; 1(7335): 702-3.

2. Rezk SA, Zhao X, Weiss LM. Epstein-Barr virus (EBV)-associated lymphoid proliferations, a 2018 update. Hum Pathol. 2018; 79: 18-41.

3. Al Hamad M, Matalka I, Al Zoubi MS, Armogida I, Khasawneh R, Al-Husaini M, et al. Human Mammary Tumor Virus, Human Papilloma Virus, and Epstein-Barr Virus Infection Are Associated With Sporadic Breast Cancer Metastasis. Breast Cancer (Auckl). 2020; 14. DOI: 10.1177/1178223420976388.

4. Chen Y, Chen X, Pan L, Huang Y, Cai Y, Li J, et al. RNA helicase DHX15 decreases cell apoptosis by NF-kappaB signaling pathway in Burkitt lymphoma. Cancer Cell Int. 2022; 22(1): 92.

5. Edwards RH, Raab-Traub N. Major Role for Cellular MicroRNAs, Long Noncoding RNAs (lncRNAs), and the Epstein-Barr Virus-Encoded BART lncRNA during Tumor Growth In Vivo. mBio. 2022; 13(3): e0065522.

6. Stanjek-Cichoracka A, Laszewska A, Wozniak-Grygiel E, Necki M, Latos M, Zawadzki F, et al. Assessment of the Serologic Status in Epstein-Barr Virus in Patients Qualified for Lung Transplantation in the First Half of 2021. Transplant Proc. 2022;54(4):1124-6.

7. Yang ZC, Nie ZQ, Chen QY, Du CC, Luo DH, Liu LT, et al. Cost-Effectiveness analysis of combining plasma Epstein-Barr virus DNA testing and different surveillance imaging modalities for nasopharyngeal carcinoma patients in first remission. Oral Oncol. 2022; 128: 105851.

8. Parker GD, Harnsberger HR, Jacobs JM. The pharyngeal mucosal space. Semin Ultrasound CT MR. 1990; 11(6): 460-75.

9. Chong VF, Fan YF. Radiology of the parapharyngeal space. Australas Radiol. 1998; 42(3): 278-83.
10. Stambuk HE, Patel SG. Imaging of the parapharyngeal space. Otolaryngol Clin North Am. 2008; 41(1): 77-101, vi.
11. Eyibilen A, Baykara M, Ozbay AS. A possible cause of snoring: bilateral tortuosity of the internal carotid artery. Kulak Burun Bogaz Ihtis Derg. 2008; 18(4): 250-2.
12. Zhou J, Jolly S. Obstructive sleep apnea and fatigue in head and neck cancer patients. Am J Clin Oncol. 2015; 38(4): 411-4.
13. Bhatt AA. Non-traumatic causes of fluid in the retropharyngeal space. Emerg Radiol. 2018; 25(5): 547-51.
14. Castellano G, Bonilha L, Li LM, Cendes F. Texture analysis of medical images. Clin Radiol. 2004; 59(12): 1061-9.
15. Tixier F, Hatt M, Le Rest CC, Le Pogam A, Corcos L, Visvikis D. Reproducibility of tumor uptake heterogeneity characterization through textural feature analysis in 18F-FDG PET. J Nucl Med. 2012; 53(5): 693-700.
16. Yang D, Rao G, Martinez J, Veeraraghavan A, Rao A. Evaluation of tumor-derived MRI-texture features for discrimination of molecular subtypes and prediction of 12-month survival status in glioblastoma. Med Phys. 2015; 42(11): 6725-35.
17. Baykara M, Koca TT, Demirel A, Berk E. Magnetic resonance imaging evaluation of the median nerve using histogram analysis in carpal tunnel syndrome. Neurological Sciences and Neurophysiology. 2018;35(3):145-50.
18. Kim KY, Le Q-T, Yom SS, Pinsky BA, Bratman SV, Ng RHW, et al. Current State of PCR-Based Epstein-Barr Virus DNA Testing for Nasopharyngeal Cancer. J Natl Cancer Inst. 2017; 109(4). DOI: 10.1093/jnci/djx007.
19. Materka A. Texture analysis methodologies for magnetic resonance imaging. Dialogues Clin Neurosci. 2004; 6(2): 243-50.
20. Baykara S, Baykara M, Mermi O, Yildirim H, Atmaca M. Magnetic resonance imaging histogram analysis of corpus callosum in a functional neurological disorder. Turk J Med Sci. 2021; 51(1): 140-7.
21. Baykara M, Baykara S. Texture analysis of dorsal striatum in functional neurological (conversion) disorder. Brain Imaging Behav. 2022; 16(2): 596-607.
22. Ruuskanen M, Irjala H, Minn H, Vahlberg T, Randen-Brady R, Hagström J, et al. Epstein-Barr virus and human papillomaviruses as favorable prognostic factors in nasopharyngeal carcinoma: A nationwide study in Finland. Head Neck. 2019; 41(2): 349-57.
23. Ai QY, King AD, So TY, Lam WKJ, Mo FKF, Tse IOL, et al. MRI of benign hyperplasia in the nasopharynx: is there an association with Epstein-Barr virus? Clin Radiol. 2020; 75(9): 711.e13-.e18.
24. Grimm JM, Schmeling DO, Dunmire SK, Knight JA, Mullan BD, Ed JA, et al. Prospective studies of infectious mononucleosis in university students. Clin Transl Immunology. 2016; 5(8): e94.
25. Lee AW, Lung ML, Ng WT, editors. Nasopharyngeal carcinoma: from etiology to clinical practice. London: Academic Press; 2019. p.1-394.

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